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Antioxidant Activity and Total Flavonoid of *Carica papaya L.* Leaves with Different Varieties, Maturity and Solvent

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ABSTRACT

Carica papaya leaves are one of the vegetables consumed by Indonesian people, especially in Java Island. Carica papaya is easy to grow in Indonesia and has many variants, so, Carica Papaya leaves is a local potent to be developed for functional food and nutraceutical. The aim of this study was to investigate antioxidant activity and total flavonoids of Carica papaya leaves with different varieties, maturity and solvent. Carica papaya leaves (CPL) was firstly extracted by methanol to select two CPLs with high antioxidant capacity and total flavonoid. The two selected CPLs were further tested with different ages mainly young and mature leaves. One selected CPL was further tested with different extraction solvents. Antioxidant activity was determined by 2.2 diphenyl-1-picrylhydrazyl, DPPH and Ferric reducing antioxidant power, FRAP. This study used five varieties of Carica papaya leaves, namely Bangkok, California, Purple, Golden and Grendel. The result showed that Golden and Grendel varieties had a higher percentage of radical scavenging property than the others, which was 78.37% and 77.40% by the DPPH method. Grendel and Purple had a higher percentage of radical scavenging property, which was 45.82 and 34.32 mmol/mg. Grendel and Purple had a higher total flavonoid property, which was 50.33 and 46.02 µg/g. Mature leaves had a higher percentage of radical scavenging property than young leaves by DPPH and FRAP methods. Mature leaves had a higher total flavonoid property than young leaves in both Grendel and Purple. Grendel had a higher antioxidant activity and a higher total flavonoid property than Purple. Grendel with water extraction had a higher antioxidant activity by DPPH and FRAP methods. The total flavonoid of Grendel papaya leaves' extract with water extraction was lower than ethanol 70% and methanol.

Keyword: *Antioxidant; Carica papaya; flavonoid; leaves*

INTRODUCTION

Carica papaya leaves included in the family *Caricaceae*. Some species of *Caricaceae* has been used as a remedy for some diseases (Munoz *et al*., 2000; Mello *et al*., 2008). *Carica papaya* leaves included in the class of vegetable foods consumed by most people of Indonesia, especially Java people. A side from being a vegetable papaya leaves is also used as a traditional herbal medicine which is believed to increase appetite and anti-malaria.

Carica papaya leaves are known to contain bioactive compounds that can increase the amount of antioxidants in the blood and reduce the level of lipid peroxidation. These compounds include papain, simopapain, cystatin, α-tocopherol, ascorbic acid, flavonoids, cyanogen, glycosides and glucosinolates (Seigler *et al*., 2002). Previous study reported that papaya leaves contain bioactive compound which have anti-cancer activity such as α-tocopherol, lycopene and benzilsotiosianat (Ching and Mohammed, 2001; van Breemen and Pajkovic, 2008; Basu and Haidar, 2008).

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Miean and Mohamed (2001) also reported that papaya shoot contain flavonoids quercetin and kaempferol.

Flavonoids in plants varies both the type and the amount depends on several factors such as variety, location of growth, the process of planting, harvesting, storage and processing conditions (Haytowitz *et al*., 2013). Antioxidant activity and bioactive compounds of plants affected by crop varieties, pre-treatment with drying, extraction method and leaves maturity (Nantitanon *et al*., 2010). Selection of solvent extraction also determine the extraction. Different solvents with different polarity can produce extract yielding different and different types of flavonoids. Research by Bimark *et al*. (2010) reported that extraction with methanol and ethanol 70% result higher yielding extract than with petroleum ether. Therefore, the aim of this study was to investigate antioxidant activity and total flavonoids of *Carica papaya* leaves with different varieties, maturity and solvent.

RESEARCH METHOD

Sample

Carica papaya leaves are used in this study were obtained from local farmers in Yogyakarta with five varieties Gold, Purple, California, Bangkok and Grendel. Sampling of *Carica papaya* leaves based on observation of the number of leaves in the tree then divided three parts. Number of papaya leaves in every tree ranged between 18-25 leaves. The calculation of the number of leaves starting from the leaves that had bloomed. Young papaya leaves taken from the third leave from above whereas mature papaya leaves taken from the seventh or eighth from above.

Research Design

This study was an experimental study with non-factorial randomized design. First, analysis and total antioxidant activity of flavonoids based on varieties. Then, analysis and total antioxidant activity of flavonoids based on maturity. The last step, analysis and total antioxidant activity of flavonoids based on solvent. In the first step using five varieties of mature papaya leaves and methanol as solvent for extraction. The second step involved two varieties of *Carica papaya* leaves were chosen at the first step and also using methanol as extraction solvent. The last step using one variety of *Carica papaya* leaves and maturity was chosen at the second step with three solvents for extraction, with water, ethanol 70% and methanol extraction. Data was presented in the form of mean + SD.

Extraction

Papaya leaves were dried in an oven of 60 °C for 3 hours 3 times then milled. One gram of dried papaya leaves milled put in 20 mL of solvent and stirred. Solvents used in this study were methanol, ethanol 70% and water. The extraction method used was microwave with the setting 4 seconds on and 60 seconds off for three times then filtered using Whatman filter paper no. 1 and then stored in a refrigerator for further testing (Victorio *et al*., 2007). The extract was not evaporated but was immediately analyzed.

DPPH Assay

Determination antioxidant activity use 2, 2-diphenyl-2-picryl-hydrazil, DPPH assay and ferric reducing antioxidant power, FRAP assay. A number of 1 g powdered leaves was extracted with 50% methanol: water. To 0.75 mL of extract sample 1.5 mL of freshly DPPH solution (20 μg mL 1) was added and stirred. The decolourizing process was recorded after 5 min of reaction at 517 nm and compared with a blank control. Antioxidant activity = [(control absorbance - sample absorbance) / control absorbance] × 100% (Taie *et al*., 2008).

FRAP Assay

Determination of antioxidant activity using FRAP assay refers to the method Vichitphan *et al*. (2007) with FeSO4.7H2O as standard. Antioxidant capacity of the samples was determined by the ability of antioxidant compounds to reduce the sample ions Fe^{3+} to Fe^{2+} (Halvorsen *et al*., 2002). FRAP reagent was prepared by mixing a solution of 0.1 M acetate buffer (pH 3.6), the solution 2,4,6-tripydyl-s-triazine, TPTZ 10 mM in 40 mM HCl as many as 0.15 grams TPTZ 10 mM dissolved in 50 mL of HCl 40 mM, and 20 mM $FeCl_3.6H_2O$ solution with a volume ratio of 10: 1: 1. A total of 50 mL and 150 mL of distilled water sample was added to the tube which already contains 1.5 mL of reagent FRAP. Solution then was incubated for 8 minutes in a dark room and the room temperature. Absorbance of the sample was measured at a wave length of 594 nm and the results are calculated in equivalent of Fe^{2+} using a standard curve equation $FeSO_4.7H_2O$ with concentration range 4-24 mol/mL.

Total Flavonoid Assay

Total flavonoids were estimated using the method of Taie *et al*. (2008).To 0.5 mL of ethanolic extract, 0.5 mL of 2% $AlCl_3$ ethanol solution was added. After 1 h at room temperature filtered then the absorbance was measured at 420 nm. Total flavonoid contents were calculated as quercetin equivalent from a calibration curve.

RESULT AND DISCUSSION

In Indonesia has developed varieties of papaya including papaya Gold, Purple, California, Bangkok and Grendel. Total flavonoids and antioxidant activity of plants influenced by several factors including varieties, maturity and solvent. Antioxidant activity and flavonoid total of *Carica papaya* leaves extract from different varieties can be seen in Table 1. Gold, Grendel and Purple papaya leaves extract have the highest antioxidant activity than California and Bangkok papaya leaves extract with DPPH and FRAP methods and significantly different ($p<0.05$). The highest total flavonoids found in Grendel and Purple papaya leaves extract significantly ($p<0.05$). Base on this result, Purple and Grendel papaya leaves extract were selected to next analysis. The next analysis was antioxidant activity and flavonoid total of *Carica papaya* leaves based on maturity.

Antioxidant activity and flavonoid total of papaya leaves extract based on maturity can be seen in Table 2. Determination of the maturity of the leaves based on the position of leaves. The young leave is located in the above while the mature leave is located in the middle. Table 2 showed antioxidant activity and total flavonoid mature papaya leaves extract both Grendel and Purple higher than young papaya leaves extract. Antioxidant activity and total flavonoid of Grendel mature papaya leaves extract higher than Grendel young, Purple young and Purple mature papaya leaves extract. So, Grendel mature variety was selected to the next analysis. The next analysis was antioxidant activity and flavonoid total based on solvent for extraction.

Antioxidant activity and flavonoid total of Grendel mature papaya leaves extract according to solvent can be seen in Table 3. The solvents used were methanol, ethanol 70% and water. Table 3 described antioxidant activity of Grendel mature papaya leaves extract with water was higher than the Grendel mature papaya leaves extract with methanol and ethanol 70%. However, total flavonoid of Grendel papaya leaves extract with water solvent lower than Grendel papaya leaves extract with methanol and ethanol 70%.

DPPH donates electrons to prevent lipid peroxidation while FRAP demonstrates the ability of the extract to convert $Fe3+$ to iron $Fe2+$ (Visavadiya *et al.,* 2009). Antioxidant activity in plants is caused by the presence of phytochemical compounds such as phenolics, anthocyanins, and flavonoid content (Cao *et al.,* 1997). Antioxidant capacity in plants is influenced by plant type, level of maturity and environmental factors such as sun exposure. Research by Kacharava *et al.* (2009) showed that irradiation can affect the level of antioxidants in cabbage and beet leaves whereas the preparation before the extraction process and the level of maturity affect the antioxidant activity in guava leaves

Table 1. Antioxidant activity and total flavonoid of *Carica papaya* leaves (CPL) extract based on varieties

Varieties	DPPH (%)	FRAP (mmol/mg)	Total flavonoid (μg/g)
California	71.15±0.00^{ab}	16.64±0.71^{a}	46.02±3.38^{ab}
Gold	78.37±3.40^{c}	24.65±4.74^{ab}	36.93±2.72^{a}
Bangkok	67.31±0.00^{a}	19.25±1.16^{ab}	50,34±6,63^{b}
Purple	74.52±2.04^{bc}	30.68±4.99^{b}	60.97±0.31^{c}
Grendel	77.40±0.68^{c}	42.18±6.72^{c}	76.69±0.13^{d}

Table 2. Antioxidant activity and flavonoid total of *Carica papaya* leaves (CPL) extract based on maturity

Maturity	DPPH (%)	FRAP (mmol/mg)	Total flavonoid (μg/g)
Grendel young	64.43±2.72^{a}	11.25±4.29^{a}	32.88±1.77^{b}
Grendel mature	77.40±0.68^{c}	42.18±6.72^{b}	76.69±0.13^{d}
Purple young	69.72±3.40^{ab}	6.50±2.53^{a}	28.28±0.89^{a}
Purple mature	74.52±2.04^{bc}	30.68±4.99^{b}	60.97±0.31^{c}

Table 3. Antioxidant activity and flavonoid total of *Carica papaya* leaves (CPL) extract based on solvent

Solvent	DPPH (%)	FRAP (mmol/mg)	Total flavonoid (µg/g)
Water	75.48±0.68[b]	60.57±2.83[b]	29.26±0.49[a]
Ethanol 70%	69.71±0.68[a]	45.68±0.05[a]	42.38±0.45[b]
Methanol	77.40±0.68[b]	42.18±6.72[a]	76.69±0.13[c]

(Nantitanon et al., 2010). The antioxidant activity of sweet potato roots and leaves was found to be different in various cultivars (Prior and Cao, 2000; Hue et al., 2012).

Flavonoids are secondary plant metabolites and are found in fruits, vegetables and some grains. The amount of flavonoids varies greatly depending on several factors such as disease, insect/pest attack, climate change, ultraviolet radiation and others (Dixon and Paiva, 1995; Winkel-Shirley, 2002). Other factors include cultivars, location of growth, agricultural practices, harvest and storage conditions as well as processing and preparation methods (Amiot *et al.,* 1995; Patil *et al.,* 1995; Hakkinen *et al.,* 2000; Van der Sluis *et al.,* 2001). Studies of flavonoids have been carried out because of its antioxidant properties that contribute to human health. Flavonoid antioxidant activity is divided into two mechanisms, namely scavenging and chelating (Cook and Samman, 1996). The structure of flavonoids has been known to contribute to the oxidative properties of the extract. Green vegetables are known to contain high antioxidant activity and are mostly derived from flavonoids (Hue *et al.,* 2012). This research proves that variety and maturity can affect antioxidant activity and total flavonoid content.

Parameters as time, solvent, temperature and extraction technique influence secondary metabolites extraction (Victorio *et al*., 2008). Solvent type and method of extraction are fundamental factors to consider for optimizing yield extraction (Goli *et al*., 2004). Methanol and ethanol 70% are a universal solvent that can dissolve compound that are polar and nonpolar. Methanol and ethanol 70% solvents produced higher extraction results than using petroleum ether. Aqueous, alcoholic and hydroalcoholic extracts are commonly used in researches with plant crude extracts (Turkmen *et al*., 2006). Water is a polar solvent and food grade, so more save than methanol and ethanol 70%. Base on the obtained result, the highest total flavonoid content was found with methanol extraction. The same result reported by Bimark *et al*. (2011) that the highest extraction yield was found with methanol solvent which extracted seven flavonoid compounds including catechin, epicatechin, rutin, myricetin, luteolin, apigenin and naringenin. Different with antioxidant activity, the highest antioxidant activity is found in papaya leaves extract with water extraction both by DPPH method and FRAP method. It may be caused by the amount of antioxidant higher than oxidant or be caused by each solvent has a deep specificity the ability to recover active components in the material.

Pearson correlation showed, there was a positive correlation relationship between flavonoid content and antioxidant activity assayed by ferric reducing antioxidant power, FRAP assay (r=0.846). Conversely, no correlation was found for antioxidant activity by DPPH radical scavenging assay. Previous study reported that antioxidant activity of plant material was very well correlated with the content of flavonoid compounds (Maisarah *et al*., 2013).

CONCLUSION

This study obtained that varieties, maturity and solvent influence antioxidant activity and total flavonoid content of *Carica papaya* leaves extract. Mature Grendel papaya leaves with water extraction have antioxidant activity higher than others but mature Grendel papaya leaves have flavonoid total lower than others.

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CONFLICT OF INTEREST

This research doesn't have conflict of interest.

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